Antigen-specific therapy for autoimmune disease

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The application of self-antigens as therapeutic tools is validated in inbred animal models of autoimmune disease. Mechanisms of antigen-induced tolerance (apoptosis, anergy, regulatory T cells and immune deviation) are being clarified in relation to the properties of antigens and the modes and routes of their delivery. Mucosa-mediated tolerance remains the predominant mode of antigen-specific therapy but awaits demonstration of clinical efficacy in human autoimmune disease.

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Abbreviations

APC	antigen-presenting cell
APL	altered peptide ligand
CTL	cytotoxic T lymphocyte
EAE	experimental autoimmune encephalomyelitis
EAU	experimental autoimmune uveitis
GA	glatiramer acetate
HA	hemagglutinin
IEL	intraepithelial lymphocyte
Ig	immunoglobulin
i.m.	intramuscular
i.p.	intraperitoneal
IRBP	interphotoreceptor retinoid-binding protein
i.v.	intravenous
LN	lymph node
MBP	myelin basic protein
OVA	ovalbumin
PLP	proteolipid protein
s.c.	subcutaneous

Introduction

Autoimmune diseases result from dysregulated immune responses to self-antigens. The presence of self-reactive lymphocytes in the blood of healthy individuals [1,2] implies that self-antigen-specific regulatory mechanisms are physiological and prevent pathological autoimmunity. Adapting the physiological ability of self-antigens to elicit homeostatic and protective immunity ('tolerance') that is targeted and free of the systemic side-effects of conventional immunosuppressive agents is the 'Holy Grail' of human autoimmune disease therapy. However, the realization that the TCR is degenerate in its recognition of antigen adds a further level of complexity.

Antigen-specific strategies (Table 1) have been shown to prevent and suppress experimental autoimmune diseases in rodents (Figure 1). Antigen-specific tolerance operates directly on effector T cells (via apoptosis or anergy) and via Tr cells that secrete anti-inflammatory cytokines or compete with effector T cells at the level of the antigen-presenting cell (APC) (Table 2). How these mechanisms of tolerance are inter-related is not completely understood. Tr cells and immune deviation could both be the net outcome of apoptosis/anergy; alternatively, Tr cells could induce anergy in effector T cells.

Table 1

Antigen-specific strategies that prevent experimental autoimmune disease.

Antigen administered via a tolerizing route
Mucosal
Dermal
Antigen administered in a tolerogenic form
Soluble (i.v. or i.p.)
Soluble peptide–MHC (i.v.)
With blockade of co-stimulation 'second signal'
As an APL
As an aggregated Ig chimera
Antigen-specific T cells administered in an immunogenic form

The nature of antigen-specific tolerance reflects a range of determinants (Table 3). Of importance clinically is the ability of Tr cells to exert antigen-non-specific 'bystander suppression' in response to specific antigen that is recognised locally at the site of the lesion or in the draining lymph node (LN) (reviewed in [3•]). Bystander suppression resolves the dilemma of multiple autoantigens in human autoimmune diseases [4], by obviating the need to know if the antigen used to induce tolerance is the major or primary pathogenic autoantigen. In contrast, tolerance based on anergy or deletion is limited to each individual antigen.

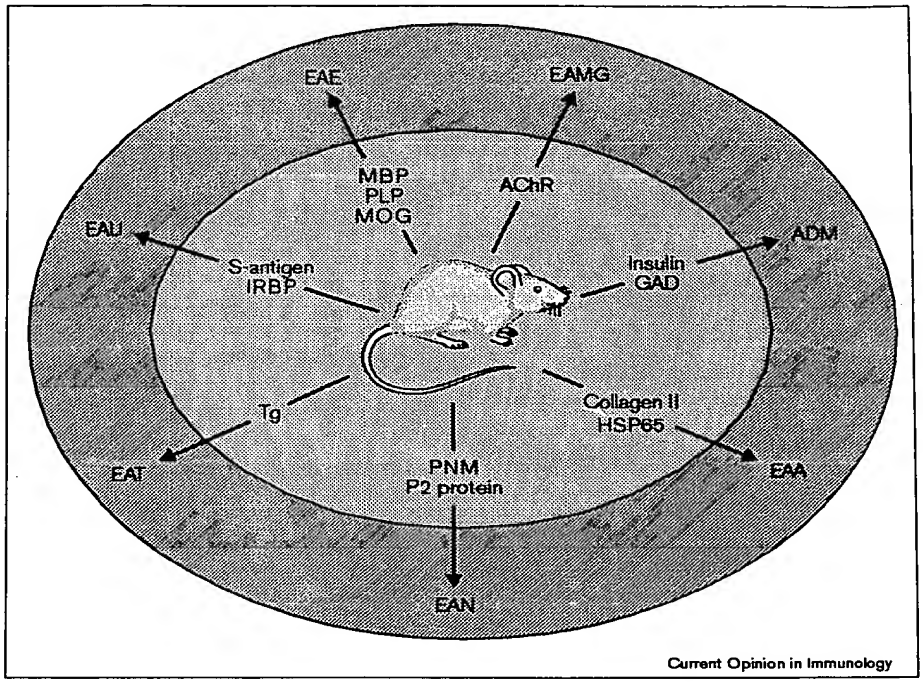
Here, the paradoxical use of self-antigens as tools for autoimmune disease therapy will be discussed. Either systemic or mucosal administration of antigen can induce tolerance based on similar mechanisms (Table 2) but the mucosal route appears more likely to induce Tr cell responses that prevent activation of Th1 autoreactive T cells.

Systemic administration of antigen

High-dose soluble peptide or monomeric protein delivered by the intraperitoneal (i.p.), subcutaneous (s.c.) or intravenous (i.v.) route induces clonal deletion or clonal anergy [5]. Systemic administration of soluble peptide–MHC complexes, including single-chain constructs in which the peptide antigen is genetically encoded within the same exon as the linked β1 and α1 MHC domains [6•] has the same effect. Generally, the immunodominance of an antigen and the affinity of a peptide epitope for MHC correlate

Figure 1

Antigen-specific tolerance in rodent models of autoimmune disease. Various antigens can be used to induce autoimmunity: MBP, PLP or myelin oligodendrocyte glycoprotein (MOG) can induce EAE; acetylcholine receptor (AChR) can induce experimental autoimmune myasthenia gravis (EAMG); insulin or glutamic acid decarboxylase (GAD) can induce autoimmune diabetes mellitus (ADM); type II collagen or heat-shock protein (HSP)65 can induce experimental autoimmune arthritis (EAA); peripheral nerve myelin (PNM) or P2 protein can induce experimental autoimmune neuritis (EAN); thyroglobulin (Tg) can induce experimental autoimmune thyroiditis (EAT); and S-antigen or IRBP can induce EAU. As detailed in the review, these antigens can also be used to induce immune tolerance in these models – depending on the dose and route of administration.



with the ability to induce tolerance via these routes. Th1 cells appear to be more sensitive to the tolerizing effect of high-dose antigen or repeated stimulation, resulting in deviation to Th2 immunity. This may explain why i.v. antigen can be followed by the appearance of cells with properties of Tr cells. Thus, a single i.v. high dose of type II collagen prevented experimental autoimmune arthritis in wild-type mice and mice transgenic for a collagen-specific TCR, and induced splenic CD4+ T cells secreting IL-4 and IL-10 [7].

If only it was always so simple! In the NOD mouse, a hierarchy of glutamic acid decarboxylase peptide epitopes given by the i.p. route in incomplete Freund's adjuvant induced Th2-type Tr cells and suppressed diabetes development, some before and some after the onset of islet pathology; this presumably reflects the precursor frequency of responding T cell clonotypes [8•]. Irrespective of acute clonal deletion of Th1 cells, long-term protection from diabetes depended on the expansion of Th2 (IL-4-secreting) cells.

CD8+ cells have a critical role in cell-mediated autoimmune diseases. To target CD8+ T cells, Bercovici *et al.* [9••] used a TCR transgenic mouse in which most CD8+ T cells expressed a TCR that is specific for the complex of influenza virus hemagglutinin $(HA)_{512-520}$ with K^d. They showed that i.v. injection of this HA peptide induced transient activation followed by apoptosis of CD8+ T cells. The same strategy was then tested in (CD8-TCR × INS-HA)F1 double-transgenic mice that also express HA in pancreatic β cells and thereby develop spontaneous diabetes. HA peptide given by the i.v. route blocked ongoing diabetes, despite the fact that pathogenic CD8+ T cells had already infiltrated the islets. There was no obvious damage secondary to CD8+ T cell recognition of peptide *in vivo*. A priority is to identify CD8+ T cell epitopes in spontaneous disease both in animal models — as recently reported for an insulin-B-chain peptide in the NOD mouse [10] — and especially in humans.

Antigen-non-specific engagement of the TCR to induce cross–reactive (more degenerate) Tr cells may be another approach; this is exemplified by a random peptide copolymer — comprising alanine, lysine, glutamic acid and tyrosine — known as glatiramer acetate (GA), Copaxone®, Cop-1 or GLAT (reviewed in [11]). Systemic GA prevents experimental autoimmune encephalomyelitis (EAE) that is induced in various animal species by purified myelin basic protein (MBP), proteolipid protein (PLP) or myelin [11] and has recently been reported to prevent experimental autoimmune uveitis (EAU) [12].

In a Phase III clinical trial, s.c. GA in patients with early multiple sclerosis (MS) decreased relapse rates and the appearance of new lesions detected by magnetic resonance imaging [13,14]. In MS patients treated daily with s.c. GA, T cell responses were examined to various peptides: GA, the immunodominant MBP_{84-102} epitope (as a model myelin antigen), combinatorial peptide libraries covering MBP_{84-102} and a random 13-mer sequence [15••]. Combinatorial peptide libraries are a powerful tool to examine TCR degeneracy;

Table 2

Mechanisms of antigen-specific tolerance.

Clonal deletion	Antigen-induced T cell apoptosis via FasL- or TNF-dependent pathways, for example induced by high-dose soluble protein or peptide given via i.p., s.c., i.v. or mucosal routes
Clonal anergy	T cell hyporesponsiveness, which probably requires continuous presence of antigen, may be associated with downregulation of surface TCR and can sometimes be overcome by IL-2, for example induced by APL or by high-dose mucosal or systemic antigen
Tr cells	Distinct subsets of T cells that secrete anti-inflammatory cytokines (Th2→IL-4, Th3→TGF-β and Tr-1→IL-10), with 'bystander' suppressor effects on APCs or other T cells, and possibly compete with effector T cells for antigen presentation, for example induced directly with low-dose mucosal antigen or as an outcome of clonal T cell deletion or anergy
Immune deviation	A shift from a pathogenic T cell response to a less pathogenic or protective T cell response, usually from Th1 (IFN-γ-mediated) to Th2 (IL-4-mediated) or Th3 (TGF-β-mediated) immunity induced in the context of autoimmune disease prevention. Immune deviation is not strictly a mechanism but more an outcome of anergy and/or Tr cell induction

Table 3

Determinants of antigen-specific tolerance.

Antigen determinants	Dose/concentration, physical form (soluble or aggregated, intact protein or peptide), chemical composition (APL), affinity for MHC molecule, content of CD4+ and CD8+ T cell epitopes, 'immunodominance', purity and contaminants (e.g. endotoxin), and adjuvants
Route of administration	
Level of co-stimulation	
Antigen-specific precursor cell frequency and duration of treatment	
Host determinants	Species, strain, age and sex
Method of assessment	Timing, tissues analysed and methods of analysis

the more peptides a T cell clone recognizes, the more degenerate its TCR. The response of over 5000 short-term T cell lines to GA or MBP was tested. GA treatment did not appear to directly affect pre-existing memory T cells and was not selectively cross-reactive with MBP but it suppressed T cell responses to GA and promoted the secretion of IL-5 and IL-13. Thus, GA induced broadly cross-reactive T cells that secreted Th2 cytokines. The demonstration that oral GA suppresses EAE in rats and mice [16] and may be more effective than MBP is of interest because oral delivery is more convenient and perhaps more effective in inducing Tr cells.

Mucosal administration of antigen

Feeding a protein antigen suppresses subsequent systemic priming to the antigen (oral tolerance); a similar phenomenon occurs after antigen delivery via naso-respiratory routes and other mucosal routes (reviewed in 3•,17,18]). Mucosal tolerance is attributed to several mechanisms (Table 2) that may overlap, one or other predominating depending on conditions, and which evolve following lymphocyte activation in the mucosal lymphoid tissues, draining LNs and even peripheral LNs and spleen [19–21,22••]. Cells that survive antigen-induced activation/apoptosis may then be anergic and/or exhibit properties of Tr cells [21,22••].

High-dose, soluble oral antigen (ovalbumin [OVA]) activates and deletes transgenic antigen-specific T cells by apoptosis locally in Peyer's patches and also in systemic lymphoid tissues [19]. Low-dose MBP activates both CD4+ and CD8+ T cells in the mucosa; the cells secrete the anti-inflammatory cytokines IL-4, IL-10 and TGF-β [23–25]. T cells primed in the mucosa may then circulate as Tr cells. CD4+ Tr cells don't appear to be unique in terms of epitope specificity, TCR usage or MHC restriction but are categorized by their cytokine production as Th2 (that produce IL-4) [26], Th3 (that produce TGF-β) [24] or Tr1 (that produce IL-10) [27]. CD8+ Tr cells are discussed below.

Antigen form and purity are important. Oral tolerance is dependent on soluble protein antigen and is abrogated if antigen is aggregated or insoluble. Oral MBP, one of the four encephalitogenic proteins within myelin, suppressed established EAE in BIO.PL mice whereas oral myelin had no effect [28•]. In contrast to CD4+ Tr cells generated in response to oral antigen, aerosol or low-dose intranasal insulin induced CD8+ γδ Tr cells in NOD mice; these cells blocked the adoptive transfer of diabetes by effector T cells [29,30••]. Following aerosol insulin (aerosol administration delivers low-dose antigen restricted to the naso-respiratory tract), γδ T cells expressing IL-10 were detected specifically in the pancreatic LNs [30••]. Thus γδ Tr cells were induced by insulin, which had to be intact but not necessarily bioactive [30••]. On the other hand, a CD4+ T cell epitope derived from insulin B chain given intranasally [31] or oral insulin [32] induced CD4+ Tr cells.

These findings imply that CD8+ γδ Tr cells recognise conformationally intact protein, in keeping with the known properties of γδ TCRs [33], and that insulin is not significantly degraded after naso-respiratory delivery, compared with oral delivery. Thus, the mucosal site to which antigen is targeted may also determine the nature of the immune response. This may relate not only to whether the antigen is degraded (e.g. after oral delivery) or absorbed (i.e. depending on dose concentration and surface area) but also to intrinsic differences between mucosal compartments.

Making sense of T cell and cytokine responses to mucosal antig ns

Although CD8+ T cells that secrete TGF-β were the first suppressor cells to be associated with oral tolerance (to MBP) [3•], subsequently CD8+ T cells were shown not to be sufficient in EAE [25] or even essential in EAU [34]

for oral tolerance. In mice deficient in $CD8^+$ cells, intranasal acetylcholine receptor, given to suppress experimental autoimmune myasthenia gravis, was associated with increased expression of TGF-β and decreased expression of IFN-γ in draining LNs; IL-4 was not detected and IL-10 not measured [35]. This study showed that nasal tolerance also did not depend on $CD8^+$ T cells and may be mediated by $CD4^+$ (TGF-β-secreting) Th3 Tr cells. Grdic *et al.* [36] found that deficiency of $CD8^+$ T cells, although not affecting systemic tolerance to fed antigen, did abolish suppression of local gut IgA that is specific for antigen, indicating that tolerance to oral antigen is compartmentalized and requires $CD8^+$ T cells for local suppression of IgA responses.

The most abundant mucosal immune cells are intraepithelial lymphocytes (IELs), the first line of cellular immune defense at mucosal surfaces. Most IELs express the $CD8\alpha\alpha$ homodimer and up to 50% express the γδ TCR. Several lines of evidence indicate that γδ IELs are involved in induction of mucosal tolerance and regulate autoimmunity [30••].

How are these findings reconciled? $CD8^+$ γδ T cells may only be required for mucosal tolerance that is induced under certain conditions. Fujihashi *et al.* [37] found that the tolerance that is associated with $CD4^+$ T cells secreting IL-10 is induced by low-dose OVA in TCRγδ-replete but not TCRγδ-deficient mice whereas tolerance to high-dose OVA is induced in both mice. That γδ T cells are involved in tolerance induction to low-dose, but not high-dose, antigen is consistent with the secretion of IL-10 by splenic T cells of recipient mice (expressing OVA-specific TCRs) that were fed low-dose, but not high-dose, OVA [38]. However, the relationship between γδ and conventional αβ T cells in (low-dose) mucosal tolerance has not been elucidated. Studies using naso-respiratory administration of insulin [29,30••] suggest that induced $CD8^+$ γδ T cells that secrete IL-10 may in turn promote the differentiation of antigen-specific Th2 $CD4^+$ αβ T cells. Finally, as indicated, $CD8^+$ γδ T cells are only likely to be induced as Tr cells in response to antigens with an intact conformation and not to degraded proteins or peptides.

Recent evidence favours IL-4 and IL-10 as predominant mediators of Tr cell effects. When insulin was conjugated with the mucosal adjuvant, cholera toxin B chain (to enhance its mucosal tolerogenicity), and given orally to NOD mice, IL-4-secreting $CD4^+$ T cells were detected in the spleen and pancreatic LNs within four hours, followed by a decrease in IFN-γ-secreting cells; TGF-β was increased only transiently in mesenteric LNs [39•]. $CD4^+$ Tr cells were cultured from pancreatic LNs of mice (in the presence of IL-4) after oral administration of porcine insulin [40••]. They secreted IFN-γ and increased amounts of IL-4 and IL-10, and suppressed diabetogenic T cell responses against an unrelated (viral) transgene-encoded antigen in β cells (an excellent example of the bystander effect) and could not be induced in mice deficient in IL-4 or Stat 6 (i.e. deficient in IL-4 signalling). Although impaired mucosal tolerance was reported in IL-4-deficient mice [41], oral tolerance could be induced with high-dose antigen [42], implying that — in contrast to Tr cells — anergy/deletion mechanisms are not IL-4 dependent.

IL-4 and TGF-β are related by the finding that IL-4 is a differentiation factor for TGF-β-secreting cells [43]. Concomitant administration of IL-4 significantly improved the ability of oral MBP_{68-86} to suppress ongoing EAE in Lewis rats; this was associated with increased secretion of IL-4, IL-10 and TGF-β, and decreased secretion of TNF-α and IFN-γ in MBP-peptide-primed LN cells [44]. Rizzo *et al.* [45•] confirmed that $CD4^+$ T cells are responsible for most of the IL-4, IL-10 and TGF-β produced in Peyer's patches after feeding C57Bl/6 mice with interphotoreceptor retinoid-binding protein (IRBP) before uveitogenic challenge with the same protein and went on to show that IL-4 and particularly IL-10 are required for the induction of oral tolerance. TGF-β expression was increased in wild-type mice after low-dose antigen but treatment with antibody to TGF-β did not abrogate tolerance induction. This suggests that, if TGF-β has a role in oral tolerance to IRBP, it is minor. This conclusion is supported by the report of oral tolerance to OVA in TGF-β1-deficient mice [46].

Using modified antigens to induce tolerance

Altered peptide ligands

TCRs interact with the peptide–MHC complexes with high specificity but surprisingly low affinity. The on/off time of TCR interaction is critical in determining which costimulatory molecules are recruited into the immunologic synapse, thus determining the functional consequences of T cell activation. This so-called 'strength of signal' can determine either full T cell activation (with induction of an inflammatory Th1 response), partial activation (with induction of a Th2 response) or T cell anergy. In an altered peptide ligand (APL), TCR contact residues in an immunodominant self-epitope are changed to impair the T cell strength of signal [47].

APLs have been applied therapeutically in animal models of autoimmune diseases and recently in a clinical trial. One APL of the encephalitogenic $PLP_{139-151}$, given with wild-type peptide to SJL/J mice, induced PLP-specific Th2 cells that secreted IL-4 and IL-10 and prevented disease. Immunization with the APL did not inhibit generation of $PLP_{139-151}$-specific T cells *in vivo* although pre-immunization protected mice from the EAE that was induced with the peptides $PLP_{178-191}$ or myelin oligodendrocyte glycoprotein $(MOG)_{92-106}$, or with mouse MBP [48]. T cell clones (specific for the APL) from animals immunized with $PLP_{139-151}$ plus APL were cross-reactive with native $PLP_{139-151}$ peptide, produced Th2/Th0 cytokines and suppressed EAE upon adoptive transfer [49]. Young *et al.* [50••] found that the therapeutic effect of T cell clones to an APL of PLP was neutralized by antibodies to the

combination of IL-4, IL-10, IL-13 and TGF-β. Together, these findings suggest that APLs do not act as antagonists *in vivo* but mediate bystander suppression, probably by the generation of Tr cells.

To demonstrate that APLs could be effective *in vivo*, TCR transgenic mice specific for the MHC class I (K^b)-restricted $OVA_{357-364}$ were used as a source of naive $CD8^+$ T cells. Variants of the peptide at TCR contact residues given by the i.v. route were shown to suppress $CD8^+$ T cell activation, IFN-γ production and cytotoxicity [51].

The real test of an APL is with a polyclonal T cell repertoire. In human autoreactive T cell clones, an APL of MBP_{85-99} peptide induced secretion of Th2 and Th3 cytokines [52]. However, although certain APLs induce T cells specific for native MBP to secrete Th2 cytokines, this is not predictable and appears to be specific for individual T cell repertoires in the outbred human population [53•]. Similarly, a panel of mouse T cell clones that recognize MBP Ac1-9 did not reliably identify an APL [54]. Nevertheless, a clinical trial was initiated with an APL of MBP_{83-99} immunodominant epitope [55]. In a preliminary analysis, APL treatment activated autoreactive MBP-specific T cells and caused a flare-up of disease in some individuals. If variable recognition of immunodominant self-epitopes can lead to activation of autoreactive T cells cross-reactive with self, then the use of APLs to treat outbred humans is problematic.

Chimeras

An immunoglobulin (Ig) chimera carrying an encephalitogenic $PLP_{139-151}$, Ig–PLP, was presented to T cells ~100-fold better than free PLP peptide and, when aggregated, induced IL-10 secretion from macrophages and dendritic cells and protected against ongoing EAE in mice [56]. The authors propose a dual mechanism for this novel strategy. First, as Ig–PLP was injected without adjuvant, its efficient presentation is probably via peripheral APCs that do not optimally express co-stimulation molecules, thereby leading to T cell anergy. Second, IL-10 release that was induced by aggregated Ig–PLP would have a bystander effect. Although neutralization of IL-10 by antibody *in vivo* abrogated protection against EAE, other evidence for these mechanisms was not provided and adoptive transfer experiments were not performed to exclude Tr cells. Because Ig–PLP contained some Ig sequences not present in the control chimera, a more appropriate control would have been an irrelevant-peptide–Ig chimera.

A soluble, dimeric chimera of peptide and MHC class II — $HA_{110-120}$ with $I\text{–}E^d$ and Fcγ2a (DEF) — was almost 100-fold more potent in stimulating cognate T cells than the wild-type HA peptide and, *in vivo*, induced Th2 differentiation of resting and activated T cells (i.e. increased IL-4, IL-10 and specific IgG_1 but decreased IL-2, specific IgG_{2a} and cytotoxic T lymphocyte [CTL] activity), in contrast to the Th1 response to the wild-type HA peptide [57]. Chimeric antigens are novel tools for the induction of potentially therapeutic Th2 immunity and further studies of the biochemical basis of their effects on T cells are awaited with interest.

Genetic approaches to antigen-specific tolerance

In contrast to antigen delivered as protein, antigen encoded as DNA has a number of potential advantages: ease of handling; stability; lack of requirement for protein purification; purity (there is no risk from contaminants in protein preparations); production of native protein (nature does the work); the potential for tissue-specific targeting; and sustained delivery with less frequent dosing.

Transgenic self-DNA

Transgenic expression of self-autoantigens on an MHC class II promoter ($I\text{–}E\alpha^k$) in APCs of the thymus and peripheral lymphoid tissues prevents autoimmune pathology and disease, providing evidence for autoantigen-mediated pathogenicity and a rationale for antigen-specific therapy [58–60].

Naked-plasmid-encoded self-DNA

Delivery of antigen as DNA has been used to prevent experimental autoimmune disease in mice but the mechanisms are not well defined. Coon *et al.* [61] reported that intramuscular (i.m.) injection of DNA encoding the insulin B chain reduced the incidence of diabetes that is triggered by lymphocytic choriomeningitis (LCMV) infection in mice expressing LCMV nucleoprotein as a transgene in their β cells. They suggested that the protective effect might be mediated by 'bystander suppressor' $CD4^+$ T cells.

Regulatory $CD4^+$ T cells that prevented both adoptive transfer of diabetes by effector T cells and cyclophosphamide-induced diabetes in NOD mice were directly identified, after intranasal administration of mouse proinsulin DNA in a cytomegalovirus-based vector (D Kramer, A Lew, LC Harrison, unpublished data). In the EAE model, a single amino acid exchange in position 79 from serine (non-self) to threonine (self) in MBP_{68-85} dramatically abrogated the protection that was induced by i.m. DNA [62]. Furthermore, protection was highly specific for MBP_{68-85} and not for a second encephalitogenic sequence, MBP_{89-101}, in Lewis rats; reciprocal results were also found. Thus, this i.m. DNA approach did not appear to be associated with bystander suppression.

Transduction of autoantigen-specific T cells

Autoantigen-specific T cells that home to the site of pathology are vehicles for delivering gene therapy (recently reviewed by Tuohy and Mathisen [63]). However, transduction of autoreactive T cells, for example with genes for anti-inflammatory cytokines, must overcome major hurdles: it must preferentially target relatively rare antigen-specific T cells and ensure that delivery is localised, tissue-specific and sufficiently sustained for lasting clinical improvement.

Self-antigen: a 'two edged sword'?

Manipulating a self-antigen requires knowledge of autoimmune disease effector mechanisms and the ability to monitor all facets of antigen-specific immunity. Antigens that contain CTL epitopes could prime CTL immunity and seriously undermine the therapeutic efficacy and safety of antigen-specific tolerance. This may explain why protection in animal models of autoimmune disease is usually only partial after administration of mucosal antigen and why, in some cases, mucosal antigens induce or exacerbate disease [64,65]. Outcomes could reflect the balance between CTL tolerance and CTL immunity. In mice bearing a transgenic TCR to OVA and transgenic OVA in pancreatic β cells, a single oral dose of OVA primed OVA-specific CTLs and caused autoimmune diabetes [66]. Subsequently, it was found that, irrespective of dose or delivery route in C57/6 mice, mucosal OVA not only induces tolerance to subsequent priming at the level of $CD4^+$ cells, B cells and $CD8^+$ T cells but also induces CTLs (A Hänninen, NR Martinez, W Heath, LC Harrison, unpublished data). These findings emphasize the need to design strategies that minimize immunity and maximize tolerance in response to mucosal antigen for the prevention of autoimmune disease.

Conclusions: closer to the Holy Grail?

Antigen-specific therapy is logically appealing and demonstrably effective in experimental autoimmune disease in inbred rodents. This proof of concept, married with safety, makes it an attractive approach for treatment of human autoimmune disease. However, this promise is yet to be realized. Clinical trials of oral myelin in multiple sclerosis, type II collagen in rheumatoid arthritis, insulin in type 1 diabetes, S-antigen in uveitis, porcine thyroglobuin in autoimmune thyroiditis and type I collagen in scleroderma — although not associated with significant systemic toxicity or apparent exacerbation of disease — have not demonstrated clinical efficacy [3•,11,17]. However, negative outcomes are not definitive and the use of peptides rather then complex antigens, nasal rather than the oral route of administration and adjuvants or other agents that enhance tolerance and decrease potential immunity may improve the efficacy of this approach in human disease.

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